

CLAIMS

1. A plant comprising a transgenic rootstock resistant to a viral disease other than by means of expression of an anti-viral protein and a scion susceptible to the viral disease, wherein the engrafted plant is resistant to said viral disease.
- 5 2. The plant according to claim 1, wherein the transgenic rootstock resistant to a viral disease comprises a nucleic acid sequence having at least 90% identity to at least one segment of the viral genome.
3. The plant according to claim 2, wherein the transgenic rootstock resistant to a viral disease comprises a DNA construct designed for generating siRNAs targeted to the at least one segment of the viral genome.
- 10 4. The plant according to claim 2, wherein the at least one segment of the viral genome encodes a viral protein or parts thereof.
5. The plant according to claim 4, wherein the viral protein is selected from the group consisting of a viral coat protein, a viral replication protein, a viral movement protein or parts thereof.
- 15 6. The plant according to claim 5, wherein the viral protein is a viral replication protein or part thereof.
7. The plant according to claim 6, wherein the transgenic rootstock is resistant to a disease caused by a soil-borne virus.
- 20 8. The plant according to claim 7, being protected from a disease caused by a soil-borne virus selected from the group consisting of nematode-transmitted viruses: Nepoviruses: Arabis mosaic virus, Grapevine fanleaf virus, Tomato black ring virus, Raspberry ringspot virus, Tomato ringspot virus, and Tobacco ringspot virus; Tobraviruses: Pea early browning virus, Tobacco rattle virus and Pepper ringspot virus; fungal-transmitted viruses: Cucumber leafspot virus, Cucumber necrosis virus, Melon necrotic spot virus, Red clover necrotic mosaic virus, Squash necrosis virus, Tobacco necrosis satellite virus, Lettuce big-vein virus, Pepper yellow vein virus, Beet necrotic yellow vein virus, Beet soil-borne virus, Oat golden stripe virus, Peanut clump virus, Potato mop top virus, Rice stripe necrosis virus, Soil-borne wheat mosaic virus, Barley mild
- 25 virus, Barley yellow mosaic virus, Oat mosaic virus, Rice necrosis
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mosaic virus, Wheat spindle streak mosaic virus and Wheat yellow mosaic virus; viruses transmitted via root wound: Tobamovirus genera: Tobacco mosaic virus, Tomato mosaic virus, Cucumber green mottle mosaic tobamovirus, Cucumber fruit mottle mosaic virus, Kyuri green mottle mosaic virus, Odontoglossum ringspot virus, Paprika mild mottle virus, Pepper mild mottle virus, Ribgrass mosaic virus and Tobacco mild green mosaic virus; and viruses transmitted by unknown rout: Watercress yellow spot virus, Broad been necrotic wilt virus, Peach rosette mosaic virus and Sugarcane chlorotic streak virus.

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9. The plant according to claim 4, wherein the at least one segment of the viral genome encodes a putative 54 kDa protein being a fragment of the replication protein of cucumber fruit mottle mosaic virus (CFMMV).

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10. The plant according to claim 9, wherein the at least one segment of the viral genome encoding a putative 54 kDa protein has the sequence set forth in SEQ ID NO:1.

11. The plant according to claim 10, being protected from a disease caused by a soil-borne virus of the tobamovirus genus.

12. The plant according to claim 11, being protected from a disease caused by CFMMV.

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13. The plant according to claim 12, being of the Cucurbitaceae family.

14. The plant according to claim 13, being a cucumber plant.

15. The plant according to claim 3, wherein the DNA construct comprises nucleic acid sequence encoding an RNA sequence that forms at least one double-stranded RNA molecule, wherein the double stranded RNA molecule mediates cleavage of the at least one segment of the viral genome.

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16. The plant according to claim 15, wherein the DNA construct comprises:

- a. at least one plant expressible promoter operably linked to;
- b. a nucleic acid sequence encoding an RNA sequence that forms at least one double stranded RNA, wherein the double stranded RNA molecule comprises a first nucleotide sequence of at least 20 contiguous nucleotides having at least 90% sequence identity to the

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sense nucleotide sequence of the target segment of the viral genome and a second nucleotide sequence of at least 20 contiguous nucleotides having at least 90% sequence identity to the complementary sequence of the sense nucleotide sequence of said target segment of said viral genome; and optionally

c. a transcription termination signal.

17. The plant according to claim 16, wherein the first and the second nucleotide sequences are separated by a spacer sequence.

18. The plant according to claim 17, wherein the spacer sequence comprises a sequence of an intron.

19. The plant according to claim 18, wherein the spacer sequence comprises an intron of the castor bean catalase gene, having the sequence set forth in SEQ ID NO:3.

20. The plant according to claim 19, wherein the DNA construct comprises the first and the second nucleotide sequences operably linked to the same promoter.

21. The plant according to claim 20, being resistant to a virus selected from the group consisting of a soil-borne virus and a virus transmitted by a vector affecting the aerial part of the plant.

22. The plant according to claim 21, wherein the soil-borne virus is selected from the group consisting of nematode-transmitted viruses: Nepoviruses: Arabis mosaic virus, Grapevine fanleaf virus, Tomato black ring virus, Raspberry ringspot virus, Tomato ringspot virus, and Tobacco ringspot virus; Tobraviruses: Pea early browning virus, Tobacco rattle virus and Pepper ringspot virus; fungal-transmitted viruses: Cucumber leafspot virus, Cucumber necrosis virus, Melon necrotic spot virus, Red clover necrotic mosaic virus, Squash necrosis virus, Tobacco necrosis satellite virus, Lettuce big-vein virus, Pepper yellow vein virus, Beet necrotic yellow vein virus, Beet soil-borne virus, Oat golden stripe virus, Peanut clump virus, Potato mop top virus, Rice stripe necrosis virus, Soil-borne wheat mosaic virus, Barley mild mosaic virus, Barley yellow mosaic virus, Oat mosaic virus, Rice necrosis mosaic virus, Wheat spindle streak mosaic virus and Wheat yellow mosaic virus; viruses

transmitted via root wound: Tobamovirus genera: Tobacco mosaic virus, Tomato mosaic virus, Cucumber green mottle mosaic tobamovirus, Cucumber fruit mottle mosaic virus, Kyuri green mottle mosaic virus, Odontoglossum ringspot virus, Paprika mild mottle virus, Pepper mild mottle virus, Ribgrass mosaic virus and Tobacco mild green mosaic virus; and viruses transmitted by unknown rout: Watercress yellow spot virus, Broad been necrotic wilt virus, Peach rosette mosaic virus and Sugarcane chlorotic streak virus.

23. The plant according to claim 21, wherein the virus transmitted by a vector affecting the aerial parts of the plant is of a family selected from the group consisting of: Caulimoviridae, Geminiviridae, Circoviridae, Reoviridae, Tartitiviridae, Bromoviridae, Comoviridae, Potyviridae, Tombusviridae, Sequiviridae, Clostroviridae and Luteoviridae; Tobamovirus, Tobravirus, Potexvirus, Carlavirus, Allexivirus, Capillovirus, Foveavirus, Trichovirus, Vitivirus, Furovirus, Pecluvirus, Pomovirus, Benyvirus, Hordeivirus, Sobemovirus, Marafivirus, Tymovirus, Idaeovirus, Ourmivirus, Umbravirus.

24. The plant according to claim 15, wherein the at least one segment of the viral genome comprises the 3' end of Zucchini Yellow Mosaic Virus (ZYMV) genome.

25. The plant according to claim 24, wherein the at least one segment of the viral genome comprises the nucleic acid sequence set forth in SEQ ID NO:2.

26. The plant according to claim 16, wherein the first nucleotide sequence comprises a nucleic acid sequence having at least 90% identity to the nucleotide sequence set forth in SEQ ID NO:2 and fragments thereof.

27. The plant according to claim 26, wherein the second nucleotide sequence comprises a nucleic acid sequence having at least 90% identity to the complement of the nucleotide sequence set forth in SEQ ID NO:2 and fragments thereof.

28. The plant according to claim 27, being resistant to a disease caused by a virus from the Potyviridae family.

29. The plant according to claim 28, being resistant to ZYMV.

30. The plant according to claim 2, wherein the nucleic acid sequence further comprises at least one expression control sequence, selected from the group

consisting of a promoter, an enhancer, a transcription factor, a splicing signal, and a termination sequence.

31. The plant according to claim 30, wherein the promoter is a constitutive promoter.

5 32. The plant according to claim 3, wherein the nucleic acid sequence further comprises a selectable marker.

33. The plant according to claim 32, wherein the selectable marker is selected from a polynucleotide sequence encoding a product conferring antibiotic resistance and a reporter gene encoding a detectable product.

10 34. The plant according to claim 30, wherein the transcription termination signal is the NOS terminator.

35. A method for producing a plant resistant to a viral disease comprising:

- a. providing a transgenic rootstock resistant to the viral disease other than by means of expression of an anti-viral protein;
 - 15 b. providing a scion susceptible to said viral disease; and
 - c. grafting the scion onto the rootstock;
- as to obtain an engrafted plant resistant to said viral disease.

20 36. The method according to claim 35, wherein the rootstock is transformed with a nucleic acid sequence having at least 90% identity to at least one segment of the viral genome as to produce a transgenic rootstock resistant to the viral disease.

37. The method of claim 36, wherein the rootstock is transformed with a DNA construct designed for generating siRNAs targeted to the at least one segment of the viral genome.

25 38. The method according to claim 36, wherein the at least one segment of the viral genome encodes a viral protein or parts thereof.

39. The method according to claim 38, wherein the viral protein is selected from the group consisting of a viral coat protein, a viral replication protein, a viral movement protein or parts thereof.

30 40. The method according to claim 39, wherein the viral protein is a viral

replication protein or part thereof.

41. The method according to claim 40, wherein the transgenic rootstock is resistant to a disease caused by a soil-borne virus.

42. The method according to claim 41 wherein the plant is protected from a disease caused by a soil-borne virus selected from the group consisting of nematode-transmitted viruses: Nepoviruses: Arabis mosaic virus, Grapevine fanleaf virus, Tomato black ring virus, Raspberry ringspot virus, Tomato ringspot virus, and Tobacco ringspot virus; Tobraviruses: Pea early browning virus, Tobacco rattle virus and Pepper ringspot virus; fungal-transmitted viruses: Cucumber leafspot virus, Cucumber necrosis virus, Melon necrotic spot virus, Red clover necrotic mosaic virus, Squash necrosis virus, Tobacco necrosis satellite virus, Lettuce big-vein virus, Pepper yellow vein virus, Beet necrotic yellow vein virus, Beet soil-borne virus, Oat golden stripe virus, Peanut clump virus, Potato mop top virus, Rice stripe necrosis virus, Soil-borne wheat mosaic virus, Barley mild mosaic virus, Barley yellow mosaic virus, Oat mosaic virus, Rice necrosis mosaic virus, Wheat spindle streak mosaic virus and Wheat yellow mosaic virus; viruses transmitted via root wound: Tobamovirus genera: Tobacco mosaic virus, Tomato mosaic virus, Cucumber green mottle mosaic tobamovirus, Cucumber fruit mottle mosaic virus, Kyuri green mottle mosaic virus, Odontoglossum ringspot virus, Paprika mild mottle virus, Pepper mild mottle virus, Ribgrass mosaic virus and Tobacco mild green mosaic virus; and viruses transmitted by unknown rout: Watercress yellow spot virus, Broad been necrotic wilt virus, Peach rosette mosaic virus and Sugarcane chlorotic streak virus.

43. The method according to claim 38, wherein the transgenic rootstock comprises a nucleic acid sequence encoding a putative 54 kDa protein being a fragment of the replication protein of cucumber fruit mottle mosaic virus (CFMMV).

44. The method according to claim 43, wherein the transgenic rootstock comprises a nucleic acid sequence encoding a putative 54 kDa protein having the sequence set forth in SEQ ID NO:1.

45. The method according to claim 44, wherein the plant is protected from a

disease caused by a soil-borne virus of the tobamovirus genus.

46. The method according to claim 45, wherein the plant is protected from a disease caused by CFMMV.

47. The method according to claim 46, wherein the plant is of the Cucurbitaceae family.

48. The method of claim 47, wherein the plant is a cucumber plant.

49. The method according to claim 37, wherein the DNA construct comprises nucleic acid sequence encoding an RNA sequence that forms at least one double-stranded RNA molecule, wherein the double stranded RNA molecule mediates cleavage of the at least one segment of the viral genome.

50. The method according to claim 49 wherein the DNA construct comprises:

- a. at least one plant expressible promoter operably linked to;
- b. a nucleic acid sequence encoding an RNA sequence that forms at least one double stranded RNA, wherein the double stranded RNA molecule comprises a first nucleotide sequence of at least 20 contiguous nucleotides having at least 90% sequence identity to the sense nucleotide sequence of the target segment of the viral genome and a second nucleotide sequence of at least 20 contiguous nucleotides having at least 90% sequence identity to the complementary sequence of the sense nucleotide sequence of said target segment of said viral genome; and optionally
- c. a transcription termination signal.

51. The method according to claim 50, wherein the first and the second nucleotide sequences are separated by a spacer sequence.

52. The method according to claim 51, wherein the spacer sequence comprises a sequence of an intron.

53. The method according to claim 52, wherein the spacer sequence comprises an intron of the castor bean catalase gene, having the sequence set forth in SEQ ID NO:3.

54. The method according to claim 53, wherein the DNA construct comprises the first and the second nucleotide sequences operably linked to the same

promoter.

55. The method according to claim 54, wherein the plant is resistant to a virus selected from the group consisting of a soil-borne virus and a virus transmitted by a vector affecting the aerial part of the plant.

56. The method according to claim 65, wherein the soil-borne virus is selected from the group consisting of nematode-transmitted viruses: Nepoviruses: Arabis mosaic virus, Grapevine fanleaf virus, Tomato black ring virus, Raspberry ringspot virus, Tomato ringspot virus, and Tobacco ringspot virus; Tobraviruses: Pea early browning virus, Tobacco rattle virus and Pepper ringspot virus; fungal-transmitted viruses: Cucumber leafspot virus, Cucumber necrosis virus, Melon necrotic spot virus, Red clover necrotic mosaic virus, Squash necrosis virus, Tobacco necrosis satellite virus, Lettuce big-vein virus, Pepper yellow vein virus, Beet necrotic yellow vein virus, Beet soil-borne virus, Oat golden stripe virus, Peanut clump virus, Potato mop top virus, Rice stripe necrosis virus, Soil-borne wheat mosaic virus, Barley mild mosaic virus, Barley yellow mosaic virus, Oat mosaic virus, Rice necrosis mosaic virus, Wheat spindle streak mosaic virus and Wheat yellow mosaic virus; viruses transmitted via root wound: Tobamovirus genera: Tobacco mosaic virus, Tomato mosaic virus, Cucumber green mottle mosaic tobamovirus, Cucumber fruit mottle mosaic virus, Kyuri green mottle mosaic virus, Odontoglossum ringspot virus, Paprika mild mottle virus, Pepper mild mottle virus, Ribgrass mosaic virus and Tobacco mild green mosaic virus; and viruses transmitted by unknown rout: Watercress yellow spot virus, Broad been necrotic wilt virus, Peach rosette mosaic virus and Sugarcane chlorotic streak virus.

57. The method according to claim 55, wherein the virus transmitted by a vector affecting the aerial parts of the plant is of a family selected from the group consisting of: Caulimoviridae, Geminiviridae, Circoviridae, Reoviridae, Tartitiviridae, Bromoviridae, Comoviridae, Potyviridae, Tombusviridae, Sequiviridae, Clostroviridae and Luteoviridae; Tobamovirus, Tobravirus, Potexvirus, Carlavirus, Allexivirus, Capillovirus, Foveavirus, Trichovirus, Vitivirus, Furovirus, Pecluvirus, Pomovirus, Benyvirus, Hordeivirus, Sobemovirus, Marafivirus, Tymovirus, Idaeovirus, Ourmivirus, Umbravirus.

58. The method according to claim 49, wherein the at least one segment of the viral genome comprises the 3' end of Zucchini Yellow Mosaic Virus (ZYMV) genome.

59. The method according to claim 49, wherein the at least one segment of the viral genome comprises the nucleic acid sequence set forth in SEQ ID NO:2.

60. The method according to claim 50, wherein the first nucleotide sequence comprises a nucleic acid sequence having at least 90% identity to the nucleotide sequence set forth in SEQ ID NO:2 and fragments thereof.

61. The method according to claim 50, wherein the second nucleotide sequence comprises a nucleic acid sequence having at least 90% identity to the complement of the nucleotide sequence set forth in SEQ ID NO:2 and fragments thereof.

62. The method according to claim 61, wherein the plant is resistant to a disease caused by a virus from the Potyviridae family.

63. The method according to claim 62, wherein the plant is resistant to ZYMV.

64. An engrafted plant produced by the method of any one of claims 35-63.

65. The plant according to claim 64, being resistant to a virus selected from the group consisting of a soil-borne virus and a virus transmitted by a vector affecting the aerial part of the plant.

66. The plant according to claim 65, being resistant to a soil-borne virus selected from the group consisting of nematode-transmitted viruses: Nepoviruses: Arabis mosaic virus, Grapevine fanleaf virus, Tomato black ring virus, Raspberry ringspot virus, Tomato ringspot virus, and Tobacco ringspot virus; Tobraviruses: Pea early browning virus, Tobacco rattle virus and Pepper ringspot virus; fungal-transmitted viruses: Cucumber leafspot virus, Cucumber necrosis virus, Melon necrotic spot virus, Red clover necrotic mosaic virus, Squash necrosis virus, Tobacco necrosis satellite virus, Lettuce big-vein virus, Pepper yellow vein virus, Beet necrotic yellow vein virus, Beet soil-borne virus, Oat golden stripe virus, Peanut clump virus, Potato mop top virus, Rice stripe necrosis virus, Soil-borne wheat mosaic virus, Barley mild mosaic virus, Barley yellow mosaic virus, Oat mosaic virus, Rice necrosis mosaic virus, Wheat spindle streak mosaic virus and Wheat yellow mosaic virus; viruses

transmitted via root wound: Tobamovirus genera: Tobacco mosaic virus, Tomato mosaic virus, Cucumber green mottle mosaic tobamovirus, Cucumber fruit mottle mosaic virus, Kyuri green mottle mosaic virus, Odontoglossum ringspot virus, Paprika mild mottle virus, Pepper mild mottle virus, Ribgrass mosaic virus and Tobacco mild green mosaic virus; and viruses transmitted by unknown rout: Watercress yellow spot virus, Broad been necrotic wilt virus, Peach rosette mosaic virus and Sugarcane chlorotic streak virus.

67. The plant according to claim 65, being resistant to a virus transmitted by a vector affecting the aerial parts of the plant of a family selected from the group consisting of: Caulimoviridae, Geminiviridae, Circoviridae, Reoviridae, Tartitiviridae, Bromoviridae, Comoviridae, Potyviridae, Tombusviridae, Sequiviridae, Clostroviridae and Luteoviridae; Tobamovirus, Tobravirus, Potexvirus, Carlavirus, Allexivirus, Capillovirus, Foveavirus, Trichovirus, Vitivirus, Furovirus, Pecluvirus, Pomovirus, Benyvirus, Hordeivirus, Sobemovirus, Marafivirus, Tymovirus, Idaeovirus, Ourmivirus, Umbravirus.